o-Chlorophenol Biodegradation by the *Brachybacterium Rhamnosum*

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Introduction

Phenolic compounds are widely distributed in the environment from various industrial as well as natural sources, for example chlorinated phenolic compounds are specifically utilized as insecticides, herbicides, detergents, solvents, wood preservatives and antimicrobial agents. Biological methods are preferable methods to treat aromatic compounds because it is economical, and there is a low possibility of the production of byproducts[1]. Several microorganisms used are usually aerobes, including *Pseudomonas putida* [2], *Ochromonas* sp[3], , *Rhodococcus* sp. [4], *Cryptococcus* sp. [4]. These aerobes are more efficient at degrading toxic compounds because they grow faster than anaerobes and usually transform organic compounds to inorganic compounds. Except for these reports above, there is almost no published information available in the literature regarding the existence of aromatic ring cleavage activities within different species of genus *Brachybacterium*.

Isolation and Identification of the Strain

In this paper, a new chlorophenol (CP)-degrading strain was isolated from return activated sludge used in the biological treatment of waste-water of Jizhuangzi wastewater plant in Tianjin. The sludge enriched on phenol was used as a source of chlorophenol-degrading bacteria. The enrichment was maintained for 20 month with bimonthly transfers of 20% inocula to fresh revised aerobic mineral medium . Cells were grown in a stirred chemostat at room temperature under stationary conditions, Approximately 10 mM phenol 0.2mM dichlorophenol as the carbon source was added every week, pH 6.5[5].

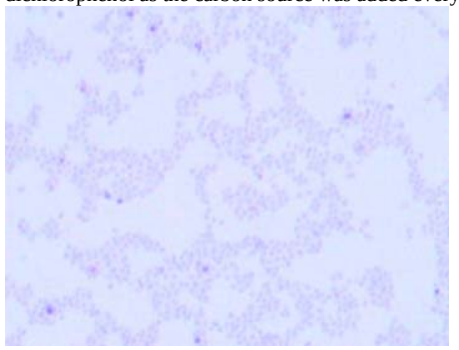

Fig 1 *Brachybacterium rhamnosum* in Microscope (magnified × 1000)

We confirmed the identification by sequencing the entire 16s rRNA gene. The 16s rDNA was amplified using a pair of forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (5'-TACGGCTACCTTGTTACGACT -3') primers following genomic DNA extraction and the corresponding PCR . 16s rDNA containing plasmid vectors were constructed using the pGEM-T Easy vector. The 16s rDNA sequences presents the strain that can be identified as *Brachybacterium rhamnosum.* This is first time to report about the *Brachybacterium rhamnosum* could be applied to degradating of chlorophenol.

Materials and Method

To clarify the level of *o*-chlorophenol(*o*-CP) biodegradation in strain *Brachybacterium rhamnosum*, this activity was investigated. Kinetic parameters for *o*-chlorophenol degradation by cells were determined by measuring *o*-CP disappearance rates. *o*-CP were determined by UV spectrophotometry monitoring changes in *o*-CP concentration, using a modified extended Kalman filter assay [6]. All spectra were recorded from 250nm to 280 nm with a 0.5 nm slit width, 100nm.min^{-1} scan speeds and very high smoothing. Calibration set with 30 standard solutions and 61 wavelengths. Standard coefficient matrix of extended Kalman filter was performed from least-squares method

Results and Discussion

The most important factor of influence reaction rate is temperature, for example, 70 mg/L *0*-chlorophenol can be reduced to 0.2 mg/L by *Brachybacterium rhamnosum* that 32 hours is needed at 32 ℃, but 72 hours is needed at 25℃. The determined rates of *o*-CP degradation were fit to the kinetics equation [5]. The whole-cell kinetic properties of *o*-CP biodegradation for strain *Brachybacterium rhamnosum* thus revealed a significant biodegradation rate. The kinetics parameters were determined from the stochastic regressive simulated through multiplicative congruence method for the equation [5]. The stochastic simulated analysis results showed that the regression relation coefficient is 0.9762 in range 25.0℃ to 32.0℃ for *o*-chlorophenol degradation. Meanwhile, the confidence level is as high as 0.9935 at the highly significance level. The confidence level is calculated through the incomplete *Beta* function, which from the definition formula of *F-distribution* function.

In conclusion, a new chlorophenol-degrading strain, *Brachybacterium rhamnosum* , was isolated from phenol-activated sludge in Tianjin. Phenotypic characteristics and phylogenetic analysis indicated the strain belong *Brachybacterium rhamnosum.* it is first time report that the *Brachybacterium rhamnosum* is being applied to taken the chlorophenol biodegradation.

References

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